

What is claimed is:

1. A method for detecting changes in gene expression of multiple distinct organisms coexisting in a sample, comprising the steps of:

- 5 (a) extracting RNA from the sample of organisms;
 (b) amplifying the RNA with and without *in vitro* RNA polyadenylation;
 (c) developing labeled probes from the amplified RNA; and
 (d) hybridizing the probes to microarrays containing the organisms to detect changes in gene expression.

10

2. A method as defined in claim 1, wherein the developing step comprises forming cDNA from the RNA and labeling the cDNA.

15 3. A method as defined in claim 1, wherein the sample of coexisting organisms is from a localized infection.

4. A method as defined in claim 3, wherein the localized infection is a murine granulomatous pouch model.

20 5. A method as defined in claim 1, wherein the sample of coexisting organisms is from a human.

6. A method as defined in claim 1, wherein the coexisting organisms comprise pathogens and hosts.

25

7. A method as defined in claim 1, further comprising repeating steps (a), (b), (c), and (d) at one or more selected time points, and comparing results from the hybridizing at each of the time points.

30 8. A method for detecting changes in gene expression of host and bacteria, comprising the steps of:

- (a) extracting a sample of localized interaction between a host and bacteria;

- (b) amplifying RNA with and without *in vitro* RNA polyadenylation; and
- (c) hybridizing said RNA to both bacteria and host microarrays to detect changes in gene expression.

- 5 9. A method as defined in claim 8, further comprising repeating steps (a), (b), and (c) at one or more selected time points, and comparing results from the hybridizing at each of the time points.
- 10 10. A method as defined in claim 8, wherein the sample of localized interaction is obtained from an animal model.
11. A method as defined in claim 10, wherein the animal model is a murine granulomatous pouch model.
- 15 12. A method as defined in claim 8, wherein the sample of localized interaction is a human sample.
13. A method for analyzing gene expression of multiple distinct organisms coexisting in a sample, comprising the steps of:
- 20 (a) obtaining a sample of a local interaction of multiple distinct organisms;
- (b) extracting RNA from said sample;
- (c) dividing said extracted RNA into a first aliquot and a second aliquot;
- (d) polyadenylating the RNA in the first aliquot;
- (e) reverse transcribing the RNA in the first aliquot and the second aliquot to
- 25 synthesize a first strand of cDNA;
- (f) synthesizing a second strand of said cDNA from the first aliquot and the second aliquot;
- (g) amplifying the RNA or cDNA;
- (h) labeling the resulting amplified product;
- 30 (i) hybridizing the resulting cDNA from the first aliquot to a microarray containing bacterial genes and the resulting cDNA from the second aliquot to a microarray containing host genes to detect gene expression; and

(j) quantifying gene expression of said bacterial genes and said host genes.

14. A method as defined in claim 13, further comprising repeating steps (a), (b), (c), (d), (e), (f), (g), (h), (i), and (j) at one or more selected time points, and comparing
5 results from the quantifying at each of the time points.

15. A method as defined in claim 13, wherein the gene expression of multiple distinct organisms coexisting in a sample is performed from the same sample of a local
infection.

10

16. A method as defined in claim 13, where the multiple distinct organisms comprise bacteria and a host.

17. A method as defined in claim 13, wherein the local interaction is an animal model
15 of local infection.

18. A method as defined in claim 17, wherein the animal model is a murine
granulomatous pouch model.

20 19. A method as defined in claim 13, wherein the sample is a human sample of local infection.

20. A method as defined in claim 13, wherein said polyadenylating is performed using
E. coli poly (A) polymerase, said synthesizing of cDNA is performed using oligo dT-
25 T7, followed by said amplifying using T7 RNA polymerase.

21. A method for evaluating differentially expressed genes comprising the steps of:
(a) hybridizing probes derived from a control homogeneous host RNA sample to a
bacterial microarray;
30 (b) hybridizing probes derived from a control homogeneous bacterial RNA sample
to an animal microarray; and

(e) detecting hybridization of said probes and eliminating cross-hybridized probes to yield a data set of differentially expressed genes for analysis.

22. A method as defined in claim 21, wherein the eliminating step comprises scaling
5 intensities from the data set to the 75th percentile and normalizing the data set, and
eliminating data for probes as cross-hybridizers where the median intensity of the
control samples after normalization is greater than 128.